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Haematological Changes in Co-Administration of Artemisinin and Anti-Oxidants to Immuno-Compromised Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author KEA designed the study. Author MAO wrote the protocol. Authors SEI managed the analyses and literature searches of the study and author EGO supervised the experimental protocol. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The health of the body is dependent on the immune system's ability to recognize and repel, or destroy foreign invaders that may cause disease. Dexamethasone (Dex), a synthetic corticosteroid medication has been known to pose immunosuppressant activity. Current study investigated the effect(s) of co-administration of Antioxidant Vitamins with Artemisinin on haematological parameters [Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Eosinophil Count (EC), Monocyte Counts (MC), Packed Cell Volume (PCV) and Lymphocyte Count (LC)] of immunocompromised mice. Sixty (n = 60) adult male (20 g- 35 g) albino mice were grouped into six (6) of ten (10) rats per group. While Group 1 received standard diet (control), Groups 2 - 6 respectively received 0.3 mg/kg body weight of Dex, 0.3 mg/kg of Dex + inoculated with *P. berghei*, 0.3 mg/kg of Dex + inoculated with *P. berghei* + 56 mg/kg of

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Artemisinin, 0.3 mg/kg of Dex + inoculated with *P. berghei* + Vitamin C, and 0.3 mg/kg of Dex + inoculated with *P. berghei* + Vit E. After 28 days of treatment, mice were fasted overnight and euthanized by cervical dislocation. Blood samples were obtained by cardiac puncture and assayed for haematological changes. Following comparison, Analysis of Variance (ANOVA) returned a statistically significant increase in Platelet Count was seen for group 3 mice, with other parameters returning an insignificant decrease. Study also found average values of PCV, LC, MCV, MCH, and MCHC to be statistically insignificant in groups 5 and 6. We recommend the co-administration of antioxidant vitamins in malaria infected and/or immunosuppressed animals.

Keywords: Malaria; anti-oxidant immunosuppressant; haematological parameters.

1. INTRODUCTION

The complex life cycle of the malaria parasites, which allows it to co-exist with the host's immune response, is largely responsible for the absence of a successful vaccine [1,2]. An ideal vaccine against malaria infection should therefore induce immune responses against every stage of the life cycle. Such as multistage, multivalent and multi-immune response vaccine presents the best strategy for successful vaccine in the treatment of malaria [3].

Though the exact mechanism of action of artemisinin derivatives had remain unknown, however some evidence point to Fenton-type reaction, generation of reactive oxygen species, alteration of mitochondrial membranes and carbon centred radical molecules that modify proteins of plasmodium parasite [4,5].

Studies have shown that co-administration of ascorbic acid (vitamin C) with dihydroartemisinin (DHA) reduced plasma ALT and AST activities in parasitized mice [6], which might be due to the protective role of ascorbic acid as water soluble free radical scavenger and antioxidant against the deleterious effects of free radicals generated by the activity of the plasmodium parasites on hepatocytes [4]. Similarly, it has been asserted that high administration of vitamin C might suppress the rate of progression of malaria parasite in infected mice [5]. However, Godswill and Olawale, [6] observed that malaria infected mice co-treated with DHA+ vitamin C had elevated plasma ALT and AST activities compared with DHA treated parasitized mice. It was hypothesized that vitamin C might have neutralized or mopped up the free radicals generated by DHA meant to be toxic to the plasmodium parasite.

Ashley, [7] in his study observed that dexamethasone and Zinc co-administration causes increase in RBC, PCV, Hemoglobin and WBC counts by possibly retarding erythrophagocytosis, and increasing erythropoietin production in the kidney [8]. With Dex known to act chiefly on certain subgroups of lymphocytes and suppressing T helper type I cell [9], it has also been shown to cause leukocytosis involving neutrophilia [10], suppression of leukocyte, blastogenesis and change T-lymphocyte subpopulation patterns [11].

In a 2016 report by Yahi et al. it was also asserted that dexamethasone causes leukocytosis in pregnant Yankasa ewes and Sahel which was mainly caused by neutrophilia as neutrophil counts were elevated in both species [12]. They observed that the increased leukocyte counts may be due to either mature neutrophils from the bone marrow storage pool, or decreased extravasation of neutrophils into the tissue. However, a decrease in the number of circulating lymphocytes in both species was also attributed (by Yahi et al.) to be due to the effects of Dexamethasone on expression of lymphocyte adhesion molecules that mediate cell to cell interactions and leukocyte extravasations. Decrease in such expression could impair lymphocyte adhesion to lymphatic vessels in tissues, with a consequent decrease of re-entry into the circulation [13].

1.1 Aim of Study

Current study examined the haematological changes in co-administration of artemisinin and anti-oxidant vitamins to immunocompromised mice. Specifically, study investigated the effect of Vitamin C and E co-administration with Artemisinin on haematological parameters in Dexamethasone (Immunosuppressed) treated mice. Study also ascertained the effect of artemisinin administration on haematological parameters of malarial infected mice.

2. MATERIALS AND METHODS

2.1 Scope of Study

Study was designed to be ex-vivo, adopting albino mice (Swiss strain) as experimental

model. The reason for choosing mice was due to the invasive approach; more so that inoculation of malaria with *P. Berghei* could not have been possible in humans. Study was conducted within the animal house of the Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria; and was limited to investigating the impact of ACT, Vitamins C and E co-administrations on haematological variables of malaria-induced mice.

2.2 Study Design

Sixty (n = 60) adult male (20 g- 35 g) albino mice of the swiss strain were used. The mice were grouped into six (6) groups of ten (10) mice per group. Group 1 received a standard rat diet *ad libitum* (control), Groups 2 - 6 respectively received 0.3 mg/kg body weight of Dex, 0.3 mg/kg of Dex + inoculated with *P. berghei*, 0.3 mg/kg of Dex + inoculated with *P. berghei* + 56 mg/kg of Artemether/Lumefantrene (A/L), 0.3 mg/kg of Dex + inoculated with *P. berghei* + Vitamin C, and 0.3 mg/kg of Dex + inoculated with *P. berghei* + Vitamin E

2.3 Inoculation with *Plasmodium berghei*

Malaria parasites, *Plasmodium berghei* were obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. Mice in the experimental group were infected by obtaining parasitized blood (3-4 drops) from the cut tail tip of the infected mice (donor). Next, 0.1ml of the collected infected blood was diluted in 0.9ml of phosphate buffer of pH 7.2 and the mice were inoculated with 0.1 ml of the parasitized blood intraperitoneally as described by David et al. [14]. This contained about twelve thousand (12000) parasites.

2.4 Determination of Parasitaemia

Parasitaemia was confirmed by preparing a thin blood film from blood obtained from the cut tail of the infected mice. This was stained with Giesma stain and viewed under the microscope. Parasitaemia was then determined by counting at least, three fields per slide with 200 TWBC per field [15];

Parasites/µL of blood = (No. of parasites x TWBC count/µL)/ 200(total leucocytes counted)

2.5 Drug Preparation and Administration

Coartem: The antimalarial drug Coartem; of the Artemether / Lumefantrine variant, Vitamin C (Ascorbic acid) and Vitamin E (α-tocopherol) were obtained from local Pharmacy store in Ekpoma, Edo State, Nigeria. The 6 tablets containing 80/480 mg/kg of both active ingredients (Atemether/Lumefantrine) were meshed into powder form and further homogenized in 150 ml of distilled water (H_20). The homogenate was then allowed to stand for 24 hours after a series of periodic stirring. The mixture was collected in a clean container and preserved in a refrigerator at minimum cool temperature. Using the orogastric cannula, 56 mg/kg (0.25 ml) of Artemisinin was administered morning and evening orally between 8:00 am and 4:00 pm for 3 days.

Vitamin C: Five hundred milligram (500 mg) of Vitamin C tablets were obtained from local pharmacy stores in Ekpoma, Edo State, Nigeria. Each tablet (500 mg) was dissolved in 100ml of distilled water, with the mixture centrifuged to obtain clear Vitamin C solution. This was then administered orally at a dose of 150 mg/kg twice daily with orogastric cannula.

Vitamin E: The tablets were dissolved in distilled water, at a dose of 150 mg/kg and administered orally via an orogastric cannula twice daily.

Dexamethasone: Dexamethasone (0.3 mg/kg) was administered once daily for 5 days with an orogastric cannula.

2.6 Samples Collection

At the end of inoculation and treatment, animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were obtained by cardiac puncture and placed in an EDTA sample container for haematological analysis.

2.7 Analysis of Haematological Parameters

Packed Cell Volume (PCV): Blood was collected and filled with heparinised capillary tube. The tube with the blood was centrifuged at a speed of 11000 revolutions per minute (rpm) for 5 minutes. RBCs packed at the bottom forms the packed cell volume with the plasma remaining above. Centrifuge was then allowed to stop automatically before reading the PCV values with the micro haematocrit reader.

Total White Blood Cell (TWBC) Count: The blood sample was diluted 1:5 with Turks solution which is 1% glacial acetic acid. With the aid of a capillary tube, the diluted sample was loaded into an improved Neuber counting chamber and the

TWBC was counted from appropriate squares in the chamber using a microscope.

Total Red Blood Cell (TRBC) Count: Here, the blood sample was diluted to 1:20 with Hayen's fluid ($HgCl_2$ 0.05 g; Na_2SO_4 2.5 g; NaCl 5 g in 100 ml of water). The diluted sample was loaded into the improved Neubaeur counting chamber with the aid of a Pasteur pipette. RBC was then counted from appropriate squares in the chamber using a microscope.

Haemoglobin Concentration: Two test tubes were labelled Test and Blank. Five millilitres (5 ml) of haemoglobin reagent was added to each test tube. 200 µ (0.02 ml) of plasma sample was then added to the test tube labelled Test and mixed properly. The solution in the test tube was then allowed to stand for 3 min at room temperature. The absorbance of the mixture was read with a spectrophotometer at 545 nm.

White Blood Cell Count: With the aid of a pasture pipette, a drop of blood was placed on a clean slide and a thin blood film was made from it. The thin film was then allowed to air dry. Next, it was stained with Leishman stain and air dried. A drop of oil immersion was placed on a stained portion of the slide and a cover slip was placed on top the oil immersion. The film was then viewed under the microscope with cells are identified and counted per field with 40x objective lens using the differential WBC counter.

Platelet Count: Platelet count was made by measuring 380µl (0.38 ml) of filtered ammonium oxalate diluting fluid into a small test tube. 20 µl (0.02 ml) of well-mixed anticoagulated blood was added and mixed thoroughly. The improved Neubaeur counting chamber was filled with the well-mixed sample and left undisturbed for 20 minutes. The underside of the chamber was dried with cotton wool and viewed under the microscope with 40x objective to count platelets that appeared as small bright fragments.

2.8 Ethical Clearance

Prior to investigation, Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State. Animals were handled in accordance with protocols approved by the institutional animal ethics committee (IAEC), as adopted by the Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria.

2.9 Statistical Analysis

Results obtained from the study were expressed as Mean ± SEM (Standard Error of Mean). With P-value of less than 0.05 ($p < 0.05$) considered to be statistically significant, a one-way analysis of variance (ANOVA) was used to determine the mean differences for variables between groups.

3. RESULTS

Results for this study are graphically presented as shown below.

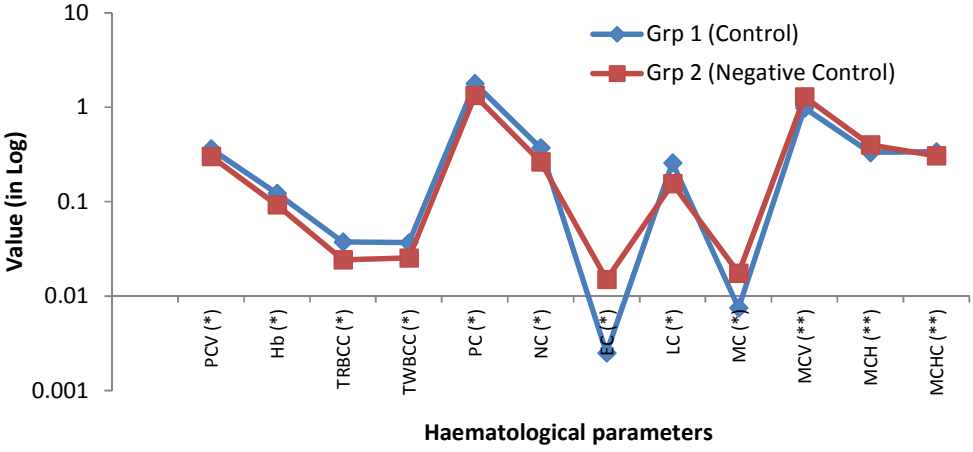

Fig. 1. Changes in haematological variables for malaria infested, untreated mice

*Grp 1: fed standard diet with no induced malaria, Grp 2: Malaria Induced, Untreated. * = significant at p ≤ .05, while **= insignificant at p > .05. Here, a statistically significant difference was observed for all but MCV, MCH and MCHC upon comparison (PCV, Hb, TRBCC, TWBCC, PC, NC, EC, LC, and MC) between groups. Values in y-axis are expressed in logarithmic form*

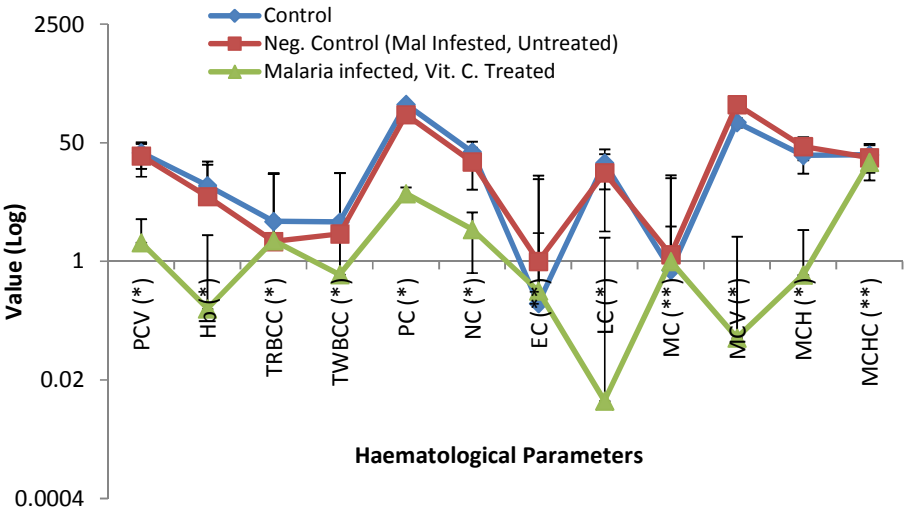

Fig. 2. Haematological changes in malaria infested, vitamin C treated mice

** = significant at p ≤ .05, while **= insignificant at p > .05. From above figure, Result showed no statistically significant difference in EC, MC, and MCHC. However, a statistically significant difference was observed for PCV, Hb, TRBCC, TWBCC, PC, NC, LC, MCV, and MCH upon comparison. Values in y-axis are expressed in logarithmic form*

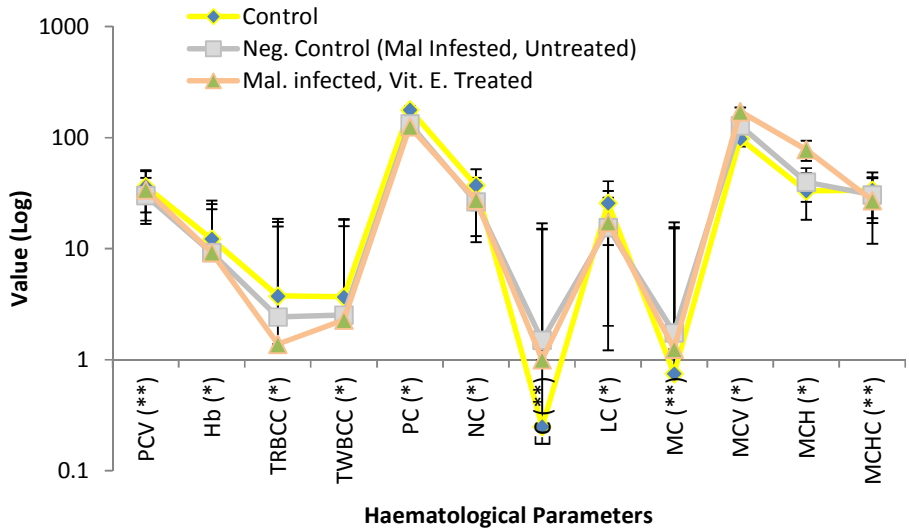

Fig. 3. Haematological changes in malaria infested, vitamin E treated mice

** = significant at p ≤ .05, while **= insignificant at p > .05. Comparison of Hematological parameters for mice of above groups showed a statistically insignificant difference for PCV, EC, MC, and MCHC, with a statistical significance observed in Hb, TRBCC, TWBCC, PC, NC, LC, MCV, and MCH. Values in y-axis are expressed in logarithmic form*

4. DISCUSSION

While blood act as a pathological reflector of the status of exposed humans to toxicant and other conditions, animals with good blood composition are likely to show good performance and/or resistance to diseases like malaria [16]. Over time, malaria infection has been associated with hematological changes and involves cells such as the red blood cells, leukocytes and

thrombocytes [17]. Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment and so could be useful in the selection of humans that are genetically resistant to certain diseases and environmental condition [16]. Haematological parameters are good indicators of the physiological status of animals, and are related to the blood and blood forming organs [18]. With conflicting reports on the effects of malaria on haematological parameters, this study was therefore devised to examine the effect of Vitamins C and E Co-Administration with Artemisinin on haematological variables in malaria infected mice.

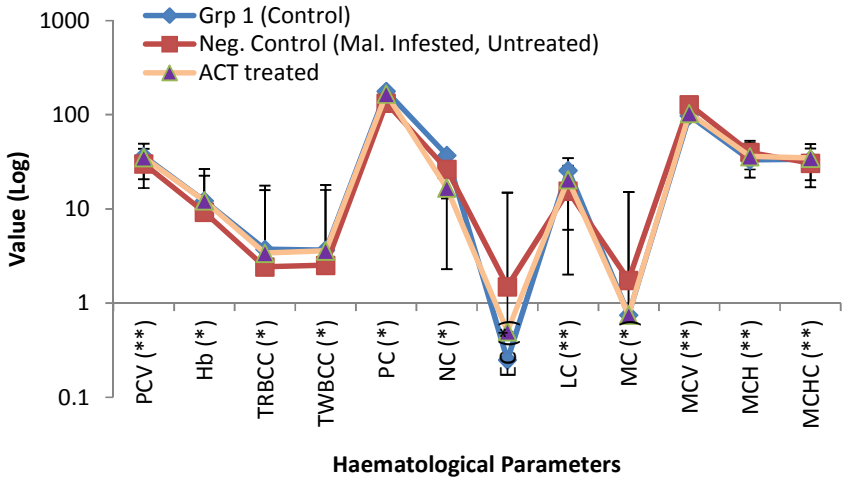

Fig. 4. Haematological changes in malaria infested, artemisinin treated mice

** = significant at p ≤ .05, while **= insignificant at p > .05. Comparison of haematological parameters for mice of above groups revealed a statistically significant difference for Hb, TRBCC, PC, NC, EC, and MC; whereas; PCV, LC, MCV, MCH, and MCHC returned a statistically insignificant difference between groups. Values in y-axis are expressed in logarithmic form*

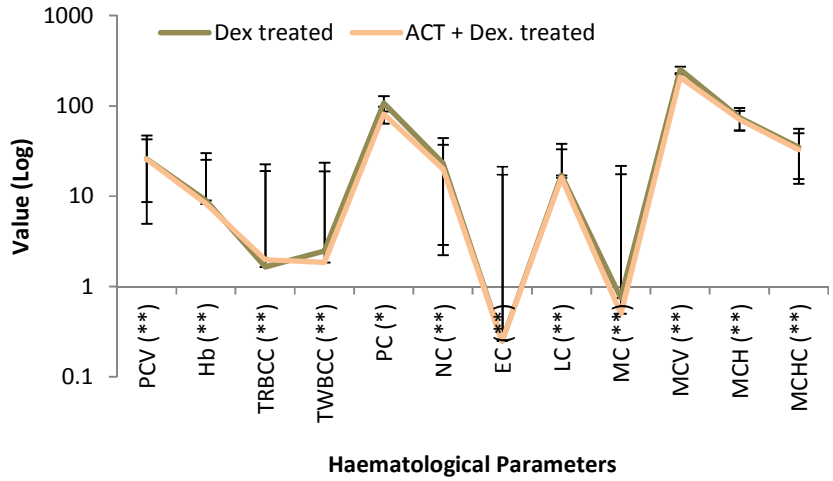

Fig. 5. Haematological changes in artemisinin treated, dexamethasone administered mice

** = significant at p ≤ .05, while **= insignificant at p > .05. Against all assayed hematological variables, above figure shows only Platelet Count (PC) with a statistically significant difference for Dexamethasone (Dex.) and ACT + Dex. Co-administered groups of malaria infested mice. All other parameters returned a statistically insignificant result, after comparing. Values in y-axis are expressed in logarithmic form*

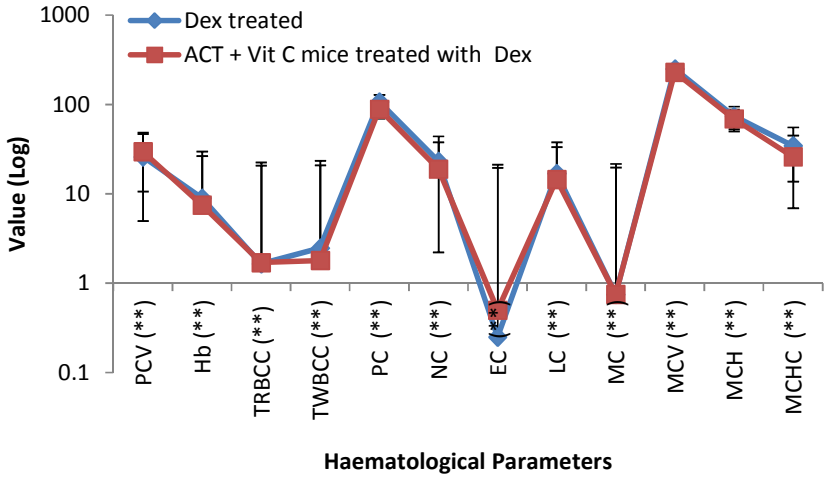

Fig. 6. Haematological changes in ACT + vitamin C treated, dexamethasone administered mice

** = significant at p ≤ .05, while **= insignificant at p > .05. From figure above, all haematological variables showed a statistically insignificant difference between groups. Values in y-axis are expressed in logarithmic form*

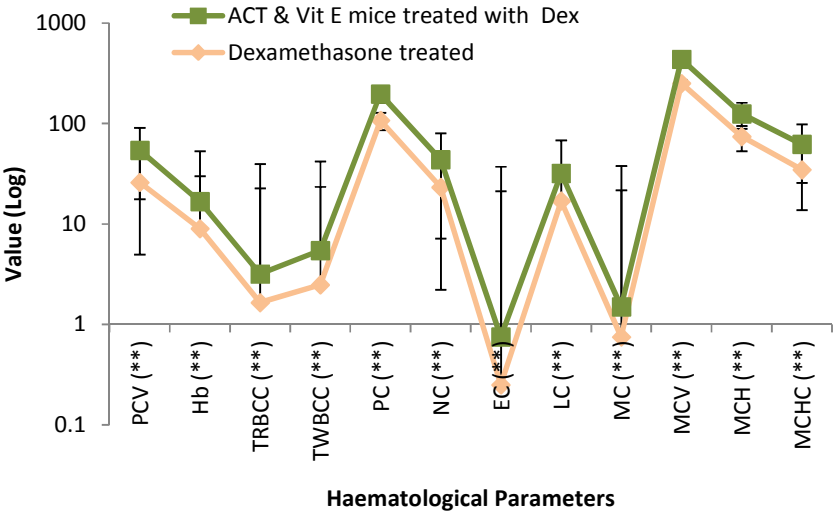

Fig. 7. Haematological changes in ACT + vitamin E treated, dexamethasone administered mice

** = significant at p ≤ .05, while **= insignificant at p > .05. Just as in Fig. 6, careful observation of figure above reveals that all haematological variables had a statistically insignificant difference (p ≤ .05) between groups on comparison. Values in y-axis are expressed in logarithmic form*

Results from present study (Fig. 1) show that compared with mice of the control group, mean packed cell volume (PCV) was significantly decreased in malaria infested, negative control mice (x = 30.25) who were left untreated than non-inoculated (control) mice (x = 36.25). This implies that on the average, malaria lowered PCV values of mice that were exposed to it. This observation is consistent with previous findings of van Wolfswinkel et al. [19] who reported malaria infected human subjects to have significantly lower Hb level than non-malaria sufferers, which ultimately results in low PCV. This is also observed to be consistent with previous studies of Sowunmi et al. [20], who reported that co-administration of Artemisinin with Vitamin E has greater effect in increasing PCV towards normalcy; an effect which may be attributed to the antioxidant capacity activity of Vitamin-E and the Artemisinin as observed by Onyesom et al. [21].

It should be stressed that reports of Maina et al. [22] that RBC counts are significantly reduced in malarial infection is consistently in agreement with findings from this present study (Fig. 1). Again, Vitamin-C and E administration in malarial infection (Figs. 5-7) showed a statistically decreased significant effect on PCV level, with no obvious increase in RBC count. However, administration of Artemisinin (Fig. 7) caused a restoration in RBC count towards normal. This corroborates with findings of previous studies that Artemisinin increases Hb and PCV values with subsequent increase in RBC count [23]. This stimulatory effect of A/L on Hb concentration and PCV levels may be beneficial in antimalarial treatment as malaria causes anaemia [24]. Another study by Obianime and Aprioku, [25] reports that Artemisinin derivatives caused an insignificant effects on RBC counts, haemoglobin and haematocrit (PCV).

Previous studies have reported a significant decrease hemoglobin (Hb) level for malarial infection in comparison to non-malaria infected patients [13]. These findings further corroborates with those of present study for each group of mice (Figs. 1-7) experimented upon. Contradicting reports from previous studies have also shown that, even though dexamethasone causes an increase in Haemoglobin level [20] by possibly retarding erythrophagocytosis, it however increases erythropoietin production in the kidney [18]. Administration of Artemisinin restored the Hb level towards normalcy; making it consistent with previous studies that showed Hb increased level following recovery from acute infections [12]. This effect of increasing Hb level by A/L is higher in mice co-administered with Vitamin-C and E; proving that Vitamin-C and E may help the body in its quest to increasing the Hb level.

As part of its research objectives, current study also investigated the changes in TWBCC variables in immunocompromised mice treated with various combinations of ACTs and Oxidative vitamins. Theoretically, White blood cells (WBCs) are the known to be the centre of target mostly by malarial infection. Ani et al. [26] had reported a significantly higher value of total WBC (leucocyte) count in malaria positive individuals as against non-malaria infected subjects. Their reports contradict findings of this study and those of Smita and Harish [27] and Igbeneghu and Odaibo [28] which showed a statistically significant decrease in mean values of total leukocyte count of malaria positive individuals. However, earlier report by Adeleye et al. [29] shows that Coartem can increase total WBC counts, which they attributed to immunological response induced by the drug at variance with the observation made by Ofem et al. [30].

5. CONCLUSION

Malaria infection is known to cause changes in haematological parameters. In this study, parasite density was seen to be reduced after the intake of Artemisinin / Lumefantrin (A/L). Treatment of malaria with A/L improved and restored deranged haematological parameters, tending it towards normal. Co-administration of A/L with vitamin-C and E had a significant potential to cause recovery from malarial infection; however, it only had effect on certain haematological parameters in this study.

6. RECOMMENDATIONS

With Anaemia being a major haematological disorder in malaria infected patients, the use of A/L therapy would lead to greater clinical and haematological benefits, following the recovery period of malaria infection. It is therefore highly recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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